Automated Deep Learning Models for the Analysis of Biological Microscopy Images
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INTRODUCTION

In almost all fields of research, including industry and academia, generating and/or analyzing data for different purposes is indispensable. Data primarily refers to a collection of numbers, audio, images, or any other type. This thesis focuses on biological data deriving from microscopes. These data are mainly images representing complex biological phenomena, such as many human diseases or quantitative traits in animals/plants [1]. Researchers can view and study micrometre-sized biological objects, such as cells and organelles, through microscopes that are invisible to the human eye [2]. A micrometre is a millionth of a meter, which is mathematically expressed as follows

\[
1 \text{ metre} = 1,000 \text{ millimetres} \\
= 1,000,000 \text{ micrometres}
\]

In simple words, one-meter lengths represented by the width of the door frame may contain 100,000 tiny cells, assuming a cell is approximately 10 micrometres.

Microscopy images are rich in information about the morphological, structural, and dynamical characteristics of tissues, cells, and molecules [2]. Observing the structural and morphological changes of the biological objects in an image helps in studying various diseases [3] [4]. The desire to observe these changes by analyzing microscopic images has increased over the years. Typical image analyses include determining the location of the biological objects, counting and clustering similar objects, computing objects’ area or volume, and other measurements [5].

Analyzing biological images suffers from various challenges:

1. In the case of possessing a large or even a small amount of microscopic images, manually analyzing each object within those images can be a tedious task.
2. Objects might overlap, which affects the accurate computation of their shape and size.
3. Image pixel intensity of the same objects might differ, depending on the type of microscope being used, the setting of configuration within the microscope being defined, and the way structures are marked in a cell.
In daily laboratory work, the analysis of microscopic images requires a lot of interactive manual work, adjusting optical and image processing parameters. Massive amounts of microscopic data in repositories can therefore not be used effectively. These challenges make it difficult to accelerate the development of the biological research domain, and thus pose a serious limitation to progress in, e.g., drug research.

Overall, microscopic images play a significant role in understanding biological processes [6], and analyzing microscopy images helps detect various diseases like breast cancer, lung cancer, and brain tumours [7]. Considering that generating and analyzing (bio)medical data remains a vital aspect of human life towards disease research and drug discovery, this thesis tries to address the three aforementioned challenges.

The remaining part of this chapter, to better understand the background of this thesis, continues by demonstrating the concept of multi-level microscopic object analysis in section 1.1, then it addresses the challenges and the requirements of researchers in biology in section 1.2 and 1.3, respectively. Afterwards, it discusses the limitations of artificial intelligence methods to contributing to the biology domain in section 1.4. Finally, it defines the research questions that introduce solutions to the issue from the AI domain despite its limitations in section 1.5.

1.1 MICROSCOPIC IMAGE COLLECTION PROCESS

Based on the object of interest, microscopic images differ. This thesis focuses on multi-level objects from organoid to cells to organelles (referred to as cell compartments). Figure 1.1 illustrates an abstract of the relationship between these three groups. In this Figure, a dome (see also Figure 6.1), coloured green, is shown, which is under the scope of a microscopic lens. The dome illustrates an organoid culture containing multiple organoids, each shown as a black bubble. An organoid consists of multiple cells, represented by yellow circles, as can be seen by zooming in on one single organoid. Finally, every cell consists of multiple organelles, for instance, Mitochondria, Nucleus, etcetera.

As defined in [8], organoids are simplified in vitro model systems of organs that are used for modelling tissue development and disease, drug screening, cell therapy, and personalized medicine. An indication for drug responses can be observed in the dynamic changes over time of organoid morphology, its size and the number of organoids in the stem [9]. More generally, analyzing organoid images involves monitoring organoids’ size and behaviour over time. As for analyzing
1.2 CHALLENGES IN BIOLOGY

The ultimate goal for the biologist is not only to study different biological phenomena under laboratory conditions but also to be able to statistically analyze all these experiments in a short time. This section provides some challenges biologists face when a quick analysis is required.

1.2.1 Manual and Limited Annotations

One of the significant challenges encountered by biologists is the limitation of annotated data. Data annotation, with respect to microscope images, is the process of labelling, more specifically tagging, relevant information for each individual object in the image for the purpose of performing biological-related analysis. An example of annotating an object, e.g. cell, would be to outline its border. The tagging of the inside area will yield a record about the objects’ condition. Even if the number of images to be analyzed can be minimal, the biggest hurdle in microscopic image analysis is the high number of objects in each image. Processing a large amount of images collected by robots employing the high-throughput imaging method within a short period of time increases the difficulty of the analysis task [5]. Typically, biologists intend to conduct and study different experimental conditions simultaneously. This can be done using multi-well plates (e.g. 96-well plate), where each well simulates an experiment.
Even though the high-throughput screening method aids biologists in thoroughly inspecting each well, analyzing all these images remains an obstacle. Considering all these obstacles, manual annotation/labelling of every object for all images in the dataset is a laborious and highly time-consuming task that also requires domain knowledge [10].

1.2.2 Manual Annotating Using Existing Software

There exist free tools that aid biologists in analyzing microscopic images. ImageJ/FIJI\(^1\), CellProfiler\(^2\) are the most used tools. Some microscope companies, such as ZEISS\(^3\), provide their own software. These interactive but commercial tools mostly carry out the analysis of the input image based on the pixel intensity distribution in the image. Images have different lighting conditions and might be taken under other microscopic parameters or even from different angles. Manual adjustment for the brightness and contrast for every single image is needed. Users must experiment with these two values to be able to analyze the content of the image, e.g. to detect objects. Thresholding based on the values from the previous step is also necessary to extract useful information. Basically, the threshold value might need to be modified from image to image since not all images have the same pixel distribution, even if captured using the same microscope. In addition to the drawback of being a manual work, the software might fail to detect objects accurately. Therefore, processing and analyzing a large amount of imaging is almost impossible using these software.

1.3 REQUIREMENTS FROM THE RESEARCHERS IN BIOLOGY

Biologists are interested in computing statistical measurements for the objects found in microscopic images. This section demonstrates their need.

1.3.1 Object Measurements

Finding and identifying particular objects present in microscopy images is the first step in the image analysis task. This includes separating objects of interest from the background. After identifying the objects, biologists are interested in the following measurements.

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1 https://imagej.net/
2 https://cellprofiler.org/
**Cell Counting**  One of the biologists’ crucial requirements is to count the number of cells seen in an image. For instance, one common indicator of harmful bodily reactions is an abnormal white blood cell count, which is essential to the body for engulfing alien objects and producing antibodies [11]. Blood cell counts assist medical professionals in making an indirect or direct pathology diagnosis [12] [13].

**Cell Condition**  Not only is cell counting essential for drug treatment, but also the number of cells for each defined group is vital to know. For example, towards improving resistance to anaemia and its diagnosis, the number of healthy and unhealthy cells is crucial to determine [14].

**Cell Type**  Classifying cells based on their types helps in understanding cellular specialization as well as organismal function and organization. Understanding the various types of neuronal cells’ contributions to brain functions is essential [15]. As stated in [16], cervical cell classification is a key technology in the intelligent cervical cancer diagnosis system. According to [17], cell type identification and discovery of subtypes have emerged as one of the most important early applications of scRNA-seq, Single-cell RNA-sequencing.

**Cell Size**  In [18], the authors give three reasons as to why do cells care how big or small they are. The fundamental functions of a cell’s physiology, such as flux across membranes, depend on the size of the cell as a whole, which is one reason why cell size matters. The basic mechanisms of cell division in eukaryotes depend on microtubules in order to form the mitotic spindle and position it correctly with respect to the cortex, which is a second factor. Third, cells must be the right size for their position within the overall tissue because, in both animals and plants, cells fit together like puzzle pieces to form tissues and organs.

Note that these measurements can be computed not only for cells but also for cell organelles or any other morphological feature.

### 1.3.2 End-to-End Systems

Referring to section 1.2.2, there exists already tools that help biologists and researchers to study and analyze microscopic images. However, these tools are based on pixel-intensity distribution and require some manual work. Therefore, a tool that can automatically analyze microscopic images is needed based on the requirements discussed in section 1.3.1 is anticipated. To put it differently, an
end-to-end system 4 is required, which covers all necessary computations from beginning to end and delivers a complete functional solution. Computers, in general, and methods from the Artificial Intelligence domain, in particular, are able to fulfil such requirements. In modern deep learning, training concerns the optimization in a processing pipeline, where all stages are simultaneously tuned according to a loss function that determines the task. This is opposed to the traditional computer vision methods that consist of a processing pipeline, where each of the individual modules needs to be manually tuned (compare section 1.2.2). The goal of this study is to design methods that can be used by non-artificial intelligence experts.

1.4 LIMITATIONS OF ARTIFICIAL INTELLIGENCE METHODS

The artificial intelligence (AI) domain offers multiple solutions to support microscopic image analysis in the biology domain. Yet, AI has its limitations, which are discussed in this section.

1.4.1 Classical Computer Vision Methods

One challenge of analyzing microscopic images arises from the fact that each type of microscope produces a different type of image depending on the study’s objectives, making these images look different. As a consequence, no unique method/technique can be applied to all kinds of microscopic images to facilitate the analysis process.

Traditional methods from the Computer Vision (CV), a subset of the artificial intelligence (AI) domain, are able to interpret visual information contained within image [19] by extracting features that help distinguish between different objects in the image [20]. Common techniques involve identifying the difference between multiple adjacent pixels in the image. Finding edges or corner points, for instance, or determining the difference in colour intensity are features that can be accomplished using Sobel [21], Canny [22], SIFT [23] and SURF [24] algorithms. The latter two algorithms compute features that are invariant against various transformations. In other words, rotating or scaling an image will result in the identical feature representation [25]. However, these feature methods are frequently sensitive to a variety of factors, including noise, light, and the

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4 The concept ‘end-to-end’ is used in two ways, in literature. In deep learning, it refers to the fact that the loss function affects all layers of a neural network. Here, we used the alternate meaning, i.e., referring to the total computation pipeline in a system.
orientation of the object in the image [25]. Training algorithms from the machine learning (ML) domains is a further step that is necessary in order to detect objects based on the extracted features. These drawbacks clearly demonstrate that CV, characterized as a manual algorithm design, can not be a dependable strategy to employ in order to overcome the challenge that various types of microscopes produce various types of images.

1.4.2 Lack of Annotated Data

Convolutional Neural Network (CNN)-based models from Deep Learning (DL), a separate branch of artificial intelligence, get around the problems discussed in 1.4.1 by being able to automatically learn features based on the entire input dataset and not being particular to one particular image. The fact that CNNs are transformation invariant as regards translation and also often scale is a huge benefit over some computer vision applications [26].

Nevertheless, CNN-based models trained via supervised technique demand a large but sufficient amount of labelled/annotated data [27]. Robots with high-throughput screening can create a large number of microscopic images. Still, those images need to be manually annotated in order to train a supervised DL model (compare section 1.2.1). For laboratories that can not afford robots, images are generated manually. Despite the fact that less data is generated in this case, the analysis of the data remains a challenge. Thus, the difficulty of automatically processing and analysing a large number of images in a short time when the corresponding annotation/labels are limited is still present.

More generally, given that deep networks use multiple deep layers of units for them to be able to extract useful features [28], classical DL models might not perform well when only a few microscope images are generated. To put it differently, deep networks require a large input dataset to ensure the development of an effective output model.

1.4.3 Microscope Resolutions

As previously mentioned in section 1.4.1, data may differ even if it is captured by the same microscope. Moreover, the same biological images might have a different resolution if taken by different microscopes. In these cases, no free-lunch theorem is applied. It describes that no single machine learning algorithm is universally performing best for all problems [29]. Transfer learning from the DL domain bypasses this issue and the insufficient amount of annotations (section 1.4.2) by training a DL model on another dataset that is rich in images and their corresponding annotations and retraining that model on the current
dataset/application [30]. This appears to be a good solution, but it can not be
depended on because it only works well only when the initial and target problems
are similar enough [31]. Hence, transfer learning can not be a trustful approach,
generally speaking.

1.4.4 Microscopic Images Differ from Natural Photographs

Natural photographs reflecting detailed and complex visual information, such
as in nature or human images, are currently available in an enormous amount
thanks to the cameras being a vital component of our life in the modern world.
Since these images are widely accessible due to the vast development of technol-
ogy, sophisticated achievements in analyzing and extracting useful information
(knowledge) from such data have already been realized: It is now possible to
detect objects, such as detecting humans and recognizing their faces [32], to
classify objects, such as pedestrians and traffic lights for self-driving cars [33], or
even to track players in football games [34], etcetera.

Contrary to such data, where objects are highly different in structure, texture,
colour and appearance, biological data are surprisingly difficult to handle. Objects
are very similar in shape, size, blurred border and structure [35]. For instance, the
peroxisome organel appears as a small roundish shape, very similar to other com-
partments present in the same cell. Furthermore, generating a massive number of
biological images has its limitations due to laboratory and professional/domain
experts’ costs. Moreover, the nature of biological images of cell/cell compart-
ments presents additional challenges because instances may partially overlap.
Its difficulty arises since instances from the same cluster type overlap. This is
different from natural photographs, where the border of overlapping objects can
be less challenging to estimate, for example, a person occluding a car, which
in turn occludes part of a house [36], [37]. The texture and color of the occclud-
ing object and its background may be highly different in the 2D image, giving
clues to what is in front and what is located towards the back, in the 3D space [38].

The majority of AI models attempt to concentrate on achieving high perfor-
mances on clean data with almost immediately distinguishable objects. Only a
small number of studies have examined overlapping objects generally. For the
biological data, it is important to precisely localize instances and their borders.
Thus, studying overlapping instances in microscopy images is a vital subject.
1.5 RESEARCH QUESTIONS

This thesis focuses on addressing and studying the main weaknesses that the current AI methods suffer from when employed to solve tasks in microscopy problems.

Part I mainly deals with approaches that can be engaged when an insufficient amount of annotated data is available. RQ1 aims at exploring the self-supervised concept and how it can be used to solve the lack of annotated data. RQ2 digs deep into the self-supervised concept and shows how complex should the pretext task be.

In Part II, it is observed that overlapping and touching objects in the general literatures have not been sufficiently addressed. Therefore, RQ3 examines the segmentation of organoid objects of microscopic images since a correct segmentation is a precondition for the subsequent biological measurements.

In Part III, an end-to-end deep-learning system is developed, in which two scenarios were studied. For practically segmenting organoid images, RQ4 demonstrates the analysis of two use cases that have been realized using the system, while RQ5 shows that the system can be used not only to segment objects but also to detect and classify them. It also manifests that the classification results can be improved by processing quarters of the image.

An explicit description of each research question is provided below:

RQ1 [Self-Supervised vs. Supervised] How can self-supervised training contribute to limited annotated biological data? (Part I, Chapter 3)

For AI to be efficiently utilized via the traditional supervised learning, raw data, along with its corresponding annotations, needs to be provided. In the case of a large amount of raw images, annotating tiny and multiple objects in every microscopic image is unrealizable. Likewise, annotating a small amount of data is not enough to build a trustworthy model due to the deep structure of any DL model. Not to mention that manually annotating microscopy images is a costly and labour task that should be avoided. That is to say, manually analyzing the rich biological information in microscopic images is challenging since it is time-consuming, it demands domain knowledge in the field, is biased to the individual human experts and therefore not entirely accurate. Therefore, it is important to find another way to automatically detect and analyze tiny objects in microscopy images while alleviating the manual functionality as much as possible. The chapter delves into the topic of creating half-way trained models that can be used to detect and segment objects when a limited amount of annotations are available.
**RQ2** [Complexity of Pretext Tasks] How complex should a pretext task for the self-supervised training learning be? (Part I, Chapter 4)

Seeing that training a deep learning model via self-supervised learning on a pretext task reduces the requirement of annotating much data (compare Chapter 3), it is, still, unclear what characteristics of the representations learned by the pretext tasks. As those learned characteristics lead to high downstream accuracies, the type and complexity of this such pretext task should be explored. An important point to consider when designing and comparing such tasks is to evaluate them under the same environment. A deeper insight should be shed on how complex the pretext tasks should be, and whether the complicity ensures a robustness performance on the main task. Investigating the type of task is still an open question. Also, the impact of training models over a range of data sizes of both labelled and unlabeled data should be tested. This chapter tries to dig into this research topic.

**RQ3** [Segmenting Overlapping Objects] How to segmented tiny and overlapped organoid object? (Part II, Chapter 5)

Most deep learning models developed to solve segmentation problems are trained and tested on clean data. Clean data indicated that objects do not touch each other or there is minimal overlap between the objects. Additionally, even if objects overlap, they can be relatively simply separated. For instance, objects in natural photographs are distinguishable in shape, structure and size, which is not the case for the biological objects in microscopic images, in which objects are very similar in morphology and they belong to the same class. Since it is essential to accurately analyze biological images, e.g. compute their sizes based on the morphology of the detected object, the outline for each object needs to be correctly estimated. In other words, when these objects overlap, some are partially or completely obstructed by others, which might result in them not being analysed and relevant information being lost along the way. This is why the detection of the overlaps is a difficult problem, especially when there is a high imbalance between classes: background, object, and overlap. The current study attempts to solve the problem of overlapping organoids in a highly imbalanced dataset.

**RQ4** [Automatization of Organoid Analysis] How can the segmentation of organoid images be automatized using an end-to-end system? (Part III, Chapter 6)
Deep learning literature is rich in methods and techniques for detecting, segmenting and classifying objects. However, these approaches remain within the group of people who are knowledgeable in deep learning and in how to make use of these techniques through extending their implementation. In particular is the topic of biological and microscopic images, where little attention is being paid to making these approaches free of access and suitable to use by non-artificial intelligence experts. Since not only automatically detecting, segmenting or even classifying objects are vital to microscopic image research, but also systematically analysing those objects plays a vital role in the progress of this area. To this end, and as a contribution to the biological domain, a free end-to-end system is desired which is able to automatically process the necessary task using AI for non-AI experts.

**RQ5** [From Segmentation to Detection and Classification] How can the end-to-end system automatically detect and classify cell compartments of fluorescent images? (Part III, Chapter 7)

Upon the success of the OrganelX e-Science service, it is vital to not to constrict this service on only one type of microscopic images. Another interesting type of images is the fluorescent microscopic images of yeast cells. Here, each image is captured in a brightfield channel as well as using a colored fluorescent protein technique. Green fluorescent protein (GFP) is one of the color transparent markers - called fluorescent labelling - that is added to cell compartments (organs). To this end, the web server is extended to handle such type of images with the goal to detect and classify tiny cell compartments in yeast cell images.

### 1.6 Outline of the Chapters

This thesis is based on several publications by the author. A list of all publications can be found on page 167. Each chapter from 3 to 7 represents one independent publication that has already been submitted and/or published, whereas chapter 2 encloses the necessary background related to this thesis. The research questions and contributions of the different topics are discussed in section 1.5.

Part I focuses on addressing the limited amount of annotated data, especially to be found in the biological microscopy images. Chapter 3 employs the self-supervised concept and compares its benefit over the traditional supervised approach in

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5 OrganelX is named for extracting (the X) organels
deep learning. Chapter 4 spotlights the first stage of the self-supervised approach by investigating the contribution of different pretext tasks to the main task and whether these tasks should be simple or complex.

In Part II, the problem of overlapping organoid objects is discussed and several approaches to overcome and solve this issue is presented.

Part III demonstrates the applications of an end-to-end system that is built during the PhD journey. These applications include the automatization of organoids analyses via segmenting raw images, as discussed in chapter 6. Chapter 7 demonstrates the capability of this system to work on cell compartment datasets, in which it atomizes the analysis to detect and classify the cell compartment objects of fluorescent images.